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Implications of BSE infection screening data for the scale of the British BSE epidemic and current European infection levels

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The incidence of confirmed clinical cases of bovine spongiform encephalopathy (BSE) in Great Britain continues to decline, but the recent discovery of cases in previously unaffected countries (including Israel, Japan, Poland, Slovenia and Spain) has heightened concerns that BSE transmission was more intense and widespread than previously thought. We use back-calculation methods to undertake an integrated analysis of data on infection prevalence in apparently healthy cattle and the incidence of confirmed clinical disease. The results indicate substantial underascertainment of clinical cases over the course of the British epidemic, and consequently that two- to fourfold more animals were infected than previously estimated. Upper bounds on the predicted size of the new variant Creutzfeldt–Jakob Disease (vCJD) epidemic are unaffected, as the prediction methods employed fit to observed vCJD mortality data, and are not sensitive to estimates of the absolute magnitude of past human exposure to BSE-infected cattle, only to relative changes in exposure through time. We also estimate the per-head incidence of infection in cattle born between 1993 and 1997 in other European Union countries, using data on the testing of apparently healthy cattle slaughtered for consumption. Infection incidence for cattle born after mid-1996 was highest in Greece, Italy and Belgium, with Spain and The Netherlands having intermediate levels, and estimates for Great Britain, Germany and France being comparably low.

Keywords: epidemiology; model; underreporting; differential mortality; subclinical infection; bovine spongiform encephalopathy

1. INTRODUCTION

Since bovine spongiform encephalopathy (BSE) was first diagnosed in England in late 1986 (Wells *et al.* 1987), more than 178 000 confirmed clinical cases have been identified in Great Britain (GB) through passive surveillance. The ban on the use of ruminant protein in the production of ruminant feed, which came into force in GB in 1988, was not completely effective, but dramatically reduced the incidence of infections (Anderson *et al.* 1996). Due to the long incubation period of BSE (5 years on average; Anderson *et al.* 1996; Ferguson *et al.* 1997) the impact of such interventions on clinical disease incidence does not become evident for some years. Thus, the annual incidence of clinical cases in GB did not peak until 1992.

Smaller epidemics occurred elsewhere in Europe, with Northern Ireland, Switzerland, the Republic of Ireland, Portugal and France reporting hundreds of clinical cases. Other countries both within the European Union (EU) and more widely (Austria, Belgium, the Czech Republic, Denmark, Finland, Germany, Greece, Israel, Italy, Japan, Liechtenstein, Luxembourg, The Netherlands, Poland, Slovakia, Slovenia, Spain) have reported small numbers of clinical cases.

Back-calculation techniques, originally developed in the early years of the HIV epidemic (Brookmeyer & Gail 1986, 1988; Isham 1989) were extended in past work for the analysis of BSE clinical incidence data (Anderson *et al.* 1996; Ferguson *et al.* 1997; Donnelly & Ferguson 2000). Back-calculation involves deconvoluting trends in disease incidence with respect to the incubation period distribution to estimate past infection incidence, while correcting for survivorship. For example, if the incubation period of a disease was known to be 3 years (with no variance) and the affected species had a probability p of surviving each year (independent of disease status), then the observation of x cases on year Y gives an estimate of x/p^3 infections in year $Y-3$. Thus, if survival is probable, then the number of infections is similar to the number of cases observed. In the case of BSE, the incubation period is long relative to the average lifespan, so the majority of infected cattle are slaughtered prior to disease onset. The situation is more complex if the incubation period is variable, as is nearly always the case. However, in such cases the back-calculation estimates of past infection incidence can still be derived and, moreover, can be used to produce predictions of future case incidence.

One of the most challenging aspects of BSE back-calculation has been modelling the rate of case ascertainment. It has been recognized throughout that, without independent data on case ascertainment rates, it is not possible to fit a time-dependent probability of case reporting across the whole epidemic (Ferguson *et al.* 1997; Donnelly & Ferguson 2000). Temporal changes in these probabilities can be estimated, but the absolute level cannot. We there-

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fore previously assumed that beyond a specific time, case ascertainment was complete, and examined the sensitivity of results to the parametric assumptions (Ferguson *et al.* 1997; Donnelly & Ferguson 2000).

It is extremely difficult to find independent data on case ascertainment rates. For scrapie, a related disease in sheep, an anonymous questionnaire was sent in 1998 to sheep farmers asking about all cases of scrapie observed in the flock over time (including those not reported to authorities) (Hoinville *et al.* 1999, 2000). On the basis of these data, it was estimated that *ca*. 13% of scrapie cases had been reported (Hoinville *et al.* 1999, 2000). Clearly, husbandry practices and species differences mean that studies of scrapie are unlikely to be a reliable indicator of BSE ascertainment. It is unlikely, even with guaranteed anonymity, that such a questionnaire would generate meaningful data given the potential consequences of not reporting BSE cases to veterinary authorities. Furthermore, given that 16% of cattle slaughtered as BSE suspects following veterinary evaluation are not confirmed histopathologically as BSE cases, it is questionable as to whether farmers would have been able to reliably identify additional BSE cases based on observation alone.

The only other potential source of information on case ascertainment is data on the prevalence of infection in slaughtered cattle. While rapid screening tests such as DELFIA (DEFRA 2001*a*) allow infection prevalence to be estimated in slaughtered cattle, interpretation of such data needs to consider the poorly characterized sensitivities of current tests, which may be low early in the long incubation period of BSE. Interpretation of large-scale screening data needs to take account of these uncertainties and to be integrated with the analysis of data on confirmed clinical cases.

The first such data available for GB result from two abattoir surveys designed to test apparently healthy cattle 'over 30 months' (OTM) of age using histopathology, Western blot, immunohistochemistry and the DELFIA test (DEFRA 2001*a*). Testing has been conducted more extensively in other European countries because current EU policy requires that all OTM cattle slaughtered for consumption must be tested for the presence of BSE infectivity, while OTM cattle have been banned for human consumption in GB since 1996. This EU requirement has resulted in the establishment of screening programmes across Europe over the last two years, with over six million cattle tested in 2001. By the end of October 2001, these active surveillance programmes have identified test-positives among apparently healthy animals (subject to normal slaughter) in Belgium, Denmark, France, Germany, Greece, the Republic of Ireland, Italy, The Netherlands, Portugal and Spain (European Commission 2002).

This paper presents methodological extensions to previously developed back-calculation techniques to allow the integrated analysis of data on clinical case incidence and results from the screening of apparently healthy cattle to obtain estimates of case ascertainment rates. We examine two different possible mechanisms underlying the apparent underascertainment of cases indicated by the British screening data: differential mortality of BSE-infected animals (suggested previously in relation to the offspring of BSE-affected dams (Donnelly *et al.* 1997*a*; Gore *et al.* 1997; Donnelly & Ferguson 2000)) and underreporting of clinical cases. For comparison, we also estimate the per-head incidence of infection, by birth cohort, in cattle in other EU countries using data on testing of apparently healthy cattle slaughtered for consumption. Though not incorporated into the analysis, consideration is given to the implications of results of other screened cattle (specifically, casualty and emergency slaughtered animals).

2. METHODS

(a) *Integrated back-calculation model*

Previous work (Anderson *et al.* 1996; Ferguson *et al.* 1997; Donnelly & Ferguson 2000) developed a back-calculation model for cohort- and age-stratified BSE clinical incidence data. If $Q(t,a)$ is the infection hazard (or force of infection; Anderson & May 1991) at time t, among susceptible individuals of age a, then the probability that an individual born at time t_{O} is infected by age a in the absence of mortality, denoted $p_{\mathrm{I}}(a|t_{\mathrm{O}})$, is given by the solution to the equation:

$$\frac{\mathrm{d}p_{\mathrm{I}}(a\,|\,t_{\mathrm{O}})}{\mathrm{d}a} = [1 - p_{\mathrm{I}}(a\,|\,t_{\mathrm{O}})]Q(t_{\mathrm{O}} + a,a). \tag{2.1}$$

Thus,

$$p_{\mathrm{I}}(A\,|\,t_{\mathrm{O}}) = \int_{\mathrm{O}}^{A} Q(t_{\mathrm{O}} + a,a)\exp\left[- \int_{\mathrm{O}}^{a} Q(t_{\mathrm{O}} + a',a')\mathrm{d}a' \right]\mathrm{d}a$$

$$= 1 - \exp\left[- \int_{\mathrm{O}}^{A} Q(t_{\mathrm{O}} + a',a')\mathrm{d}a' \right]. \tag{2.2}$$

Assuming that the dependence of exposure and susceptibility to infection on age do not vary over time, we factorize the infection hazard into the sum of products of univariate functions in time and age: the former representing a time-dependent risk of infection and the latter representing an age-dependent susceptibility/exposure distribution such that

$$Q(t,a) = \sum_{j} r_j(t)g_j(a), \tag{2.3}$$

where j indexes transmission route. BSE has three potential transmission routes: indirect horizontal via meat-and-bonemeal-containing feed (F), maternal (M) and direct horizontal (H). In each case, $g_j(a)$ is normalized over the age range 0 to 18 with $g_{\mathrm{M}}(a) = \delta(a)$, the Dirac delta function, assuming all maternal transmission occurs at the time of birth. The functional form chosen for $g_{\mathrm{F}}(a)$, defined by the cumulative density function

$$\int_{\mathrm{O}}^{a} g_{\mathrm{F}}(a')\mathrm{d}a' = (1 - \exp[-(\gamma_1 a)^{\gamma_2}])(1 - \exp[-(\gamma_3 a)^{\gamma_2+\gamma_4}]), \tag{2.4}$$

was derived empirically to be extremely flexible and was shown to provide a good fit to the cohort- and age-stratified incidence of clinical cases (Anderson *et al.* 1996; Ferguson *et al.* 1997; Donnelly & Ferguson 2000).

In this paper, we consider only feed-borne and maternal transmission as earlier analyses have demonstrated that horizontal transmission, if it occurs at all, does not contribute substantially to the transmission dynamics of the epidemic (Ferguson *et al.* 1999; Donnelly & Ferguson 2000). For additional detail on the dependence of $r_{\mathrm{M}}(t)$ on dam infectiousness over the course of the incubation period and dam demography see Ferguson *et al.*

(1997) and Donnelly & Ferguson (2000). Thus, the probability of infection by age A in an individual born at time t_0, in the absence of mortality, can be written as

$$p_I(A|t_0) = \int_0^A \left(\sum_j r_j(t_0 + a) g_j(a)\right) \times \exp\left(-\int_0^a \sum_j r_j(t_0 + a') g_j(a') \mathrm{d}a'\right) \mathrm{d}a. \quad (2.5)$$

Then, in the absence of mortality, the probability density function (PDF) that an individual born at time t_0 becomes infected at age a and a case at age u is

$$\rho_C(a,u|t_0) = \left(\sum_j r_j(t_0 + a) g_j(a)\right) \times \exp\left(-\int_0^a \sum_j r_j(t_0 + a') g_j(a') \mathrm{d}a'\right) f(u - a), \quad (2.6)$$

where $f(u)$ is the PDF for the incubation period (assuming there is no dependence on the route of infection).

However, because the incubation period of BSE is long relative to mean life expectancy of cattle, it is critical to incorporate survivorship when calculating expected case incidence. In past work (Anderson *et al.* 1996; Donnelly *et al.* 1997*a*; Ferguson *et al.* 1997, 1999; Donnelly & Ferguson 2000), we assumed that the probability of survival, excluding mortality directly attributed to clinical BSE, did not depend upon infection status. Here, we generalize the model to allow for differential mortality. Let $\mu(a)$ represent the hazard of death for an uninfected animal at age a, and let $\kappa(w)$ represent the additional mortality hazard experienced by BSE-infected cattle, prior to the onset of overt clinical signs of disease, as a function of the time until clinical onset of disease, w. The probability that an uninfected animal survives to age a, $S(a)$, is related to the mortality hazard by

$$S(a) = \exp\left(-\int_0^a \mu(a') \mathrm{d}a'\right). \quad (2.7)$$

Thus, including mortality, the probability that an individual born at time t_0 experiences clinical onset of disease by age U is

$$p_C(U|t_0) = \int_0^U \int_0^u \exp\left(-\int_0^u \mu(a'') \mathrm{d}a'' - \int_0^{u-a'} \kappa(w) \mathrm{d}w\right) \times \rho_C(a',u|t_0) \mathrm{d}a' \mathrm{d}u, \quad (2.8)$$

where the subscript C denotes the link to clinical cases.

The available data on clinical case incidence relate to confirmed cases reported as suspects to the Department for Environment, Food and Rural Affairs (DEFRA, formerly the Ministry of Agriculture, Fisheries and Food). Thus, any underascertainment of clinical cases needs to be accounted for in the model fitted to the data. Let $\Lambda(t)$ be the probability that a clinical case with onset at time t was reported to authorities. Thus, the probability that an individual born at time t_0 became a case by age U and was reported to DEFRA is

$$p_{RC}(U|t_0) = \int_0^U \Lambda(t_0 + u) \int_0^u \exp\left(-\int_0^u \mu(a'') \mathrm{d}a'' - \int_0^{u-a'} \kappa(w) \mathrm{d}w\right) \times \rho_C(a',u|t_0) \mathrm{d}a' \mathrm{d}u, \quad (2.9)$$

where the subscript RC denotes the link to reported clinical cases.

We focus on models where the excess mortality hazard is concentrated near the end of the incubation period. In the special case that a fraction K of infected animals that survive to disease onset are slaughtered just before clinical signs become apparent (i.e. $w \to 0+$), equation (2.9) becomes

$$p_{RC}(U|t_0) = (1 - K) \int_0^U \Lambda(t_0 + u) S(u) \int_0^u \rho_C(a',u|t_0) \mathrm{d}a' \mathrm{d}u. \quad (2.10)$$

This expression makes clear how the level of underreporting is confounded with the extent of differential mortality as well as the infection hazard.

The reported clinical incidence data, stratified by age, arise from a multinomial distribution and the likelihood of the data can be written in terms of $p_{RC}(U|t_0)$. The total number of animals born in the time interval t_0 to $t_0 + \Delta$, N_{t_0}, and the time-dependent birth rate, $B(t)$, were obtained from analysis of annual agricultural census data (Department of Agriculture and Fisheries for Scotland 1975–1980, 1981–1990; Ministry of Agriculture, Fisheries and Food 1975–1990, 1992–1995; Scottish Office 1991–1995) and data on the seasonality of births (Donnelly *et al.* 1997*b*). Thus, ignoring additive constants, the log likelihood of the clinical case incidence data can be written as

$$l_C = \sum_{t_0} \left(N_{t_0} - \sum_{i=1}^{i_{\max}(t_0)} X_{i,t_0}\right) \ln\left(1 - \frac{\int_{t_0}^{t_0+\Delta} B(t) p_{RC}\left(U_{i_{\max}(t_0)}|t\right) \mathrm{d}t}{N_{t_0}}\right) + \sum_{i=1}^{i_{\max}(t_0)} X_{i,t_0} \ln\left(\frac{\int_{t_0}^{t_0+\Delta} B(t)(p_{RC}(U_i|t) - p_{RC}(U_{i-1}|t))\mathrm{d}t}{N_{t_0}}\right), \quad (2.11)$$

where X_{i,t_0} is the number of confirmed cases among calves born between t_0 and $t_0 + \Delta$ with clinical onset between ages U_i and U_{i-1} from $U_0 = 0$ up to the cohort-specific maximum observable age of onset $U_{i_{\max}(t_0)}$.

The OTM surveys were carried out among a random sample of animals over 5 years of age slaughtered at abattoirs. To model these data, it is necessary to consider the slaughter rates of infected and uninfected animals. The age-specific rate at which uninfected animals, born at time t_0, are slaughtered is

$$Z_U(a|t_0) = B(t_0)\mu(a)\exp\left(-\int_0^a \mu(a'') \mathrm{d}a''\right)[1 - p_I(a|t_0)]. \quad (2.12)$$

If the diagnostic test (or combination of tests) used is not fully specific, say with specificity ξ, then a proportion, $1 - \xi$, of these uninfected animals would be detected as false positives.

The age-specific rate at which apparently healthy infected animals, born at time t_0, are slaughtered requires correction for disease-related mortality such that

$$Z_I(a|t_0) = B(t_0) \int_0^\infty \int_0^a [\mu(a) + \kappa(w)] \exp\left(-\int_0^a \mu(a'') \mathrm{d}a'' - \int_w^{w+a-a'} \kappa(w') \mathrm{d}w'\right) \rho_C(a',a + w|t_0) \mathrm{d}a' \mathrm{d}w, \quad (2.13)$$

including animals slaughtered due to both baseline and differential mortality hazards.

However, only a fraction of the apparently healthy infected animals will be detectable by an imperfect test. If we assume that the sensitivity of the diagnostic test (or combination of tests)

depends only on the time to clinical onset, the age-specific rate at which clinically unaffected infected animals are detected by the test as positive, $Z_{ID}(a \mid t_O)$, is given by

$$Z_{ID}(a|t_O) = B(t_O)\int_O^\infty \int_O^a \psi(w)[\mu(a) + \kappa(w)] \times \exp\left(-\int_O^a \mu(a'')\mathrm{d}a'' - \int_w^{w+a-a'} \kappa(w')\mathrm{d}w' \right) \times \rho_C(a', a + w \mid t_O)\mathrm{d}a'\mathrm{d}w, \quad (2.14)$$

where $\psi(w)$ denotes the sensitivity of the diagnostic test for a time w from disease onset. Thus, if a fully sensitive test is used (i.e. $\psi(w) = 1$ for all w), then $Z_{ID}(a \mid t_O) = Z_I(a \mid t_O)$.

Returning to the special case where a fraction K of infected animals that survive to disease onset are slaughtered just before clinical signs become apparent, equation (2.14) becomes

$$Z_{ID}(a|t_O) = B(t_O)S(a)\left[\mu(a)\int_O^\infty \int_O^a \psi(w)\rho_C(a', a + w \mid t_O)\mathrm{d}a'\mathrm{d}w + K\psi(0)\int_O^a \rho_C(a', a \mid t_O)\mathrm{d}a' \right]. \quad (2.15)$$

The OTM testing data of animals born between t_O and $t_O + \Delta$ and tested at age a are binomial in form. Ignoring additive constants, the log likelihood of these data can be written as

$$l_O = \sum_{t_0} (n_{t_0}(a) - x_{t_0}(a))\ln\left(1 - \frac{\int_{t_0}^{t_0+\Delta} (1-\xi)Z_U(a|t) + Z_{ID}(a|t)\mathrm{d}t}{\int_{t_0}^{t_0+\Delta} Z_U(a|t) + Z_I(a|t)\mathrm{d}t} \right) + x_{t_0}(a)\ln\left(\frac{\int_{t_0}^{t_0+\Delta} (1-\xi)Z_U(a|t) + Z_{ID}(a|t)\mathrm{d}t}{\int_{t_0}^{t_0+\Delta} Z_U(a|t) + Z_I(a|t)\mathrm{d}t} \right), \quad (2.16)$$

where $n_{t_0}(a)$ is the number of animals born between t_O and $t_O + \Delta$ tested for infection, $x_{t_0}(a)$ is the number of those which tested positive, and ξ is the specificity of the test (or combination of tests) used. We assume specificity does not depend on the age of the animal tested or on the time of testing.

Given the limited information available about test sensitivity and specificity, analyses are conducted under a range of assumptions regarding differential mortality and underreporting. Both differential mortality and underreporting act to reduce the proportion of infected animals being confirmed as clinical cases, correspondingly increasing the back-calculation estimates of past infection incidence to explain the observed incidence of clinical cases. The key difference is that the unreported clinical cases are assumed not to have entered the survey population (because only apparently healthy animals were surveyed), whereas the late-stage animals slaughtered preferentially, for example due to reduced productivity not recognized as clinical signs of BSE, were apparently healthy and thus assumed to have entered the survey population. In terms of historical food-borne exposure of consumers to BSE, animals preferentially slaughtered before clinical signs were obvious would have probably entered the food supply. Unreported clinical cases that were sent to the abattoir (as all survey animals were) would have been less likely to have passed inspections and been classified as fit for human consumption, though this possibility can not be excluded.

Maximum-likelihood estimates for the following parameters were obtained by numerical maximization of the sum of the clinical case data and OTM testing data log likelihoods, $l_C + l_O$: the time-dependent risk of feed-borne infection, $r_F(t)$; the probability of maternal transmission parameterized as a function of the time v from birth of the calf until the onset of clinical signs in the dam; the age-dependent susceptibility/exposure distribution for feed-borne risks, $g_F(a)$; the incubation period distribution, $f(u)$; the probability that a case is reported, $\Lambda(t)$; and the fraction of infected animals that survive to disease onset are slaughtered just before clinical signs become apparent, K. Demographic parameters, specifically the probability of survival, $S(a)$, and the number of calves born each month, were estimated from independent data (see details below).

(b) *Model for EU testing data*

To place these results within the wider European context, we also analysed the data arising from EU-wide testing of apparently healthy OTM cattle slaughtered for consumption. The corresponding clinical case data were not analysed, as in several countries case ascertainment was at very low levels until recently and whole-herd slaughter policies complicate analysis of case-incidence data. For country E, let $n_{t_O,E}(a)$ be the number of animals born at time t_O tested for infection at age a and $x_{t_O,E}(a)$ be the number of those which tested positive. If the test used is highly specific, then the simple ratio $x_{t_O,E}(a)/n_{t_O,E}(a)$ underestimates the incidence of infection in the cohort of animals born at time t_O, because it does not correct for either the sensitivity of the diagnostic test used or the disease-related mortality between birth and the age at which animals are tested.

We simplify the framework developed in the previous section for the analysis of testing data alone. In particular, we assume no additional mortality hazard is experienced by BSE-infected cattle prior to the onset of overt clinical signs of disease, i.e. $\kappa(w) = 0$ for all w, to obtain conservative estimates of infection incidence. Furthermore, assuming that (i) virtually all infections in animals born at time t_O occurred prior to the age of testing, a, (i.e. $p_{I,E}(a \mid t_O) \approx p_{I,E}(\infty \mid t_O) = p_{I,E}(t_O)$ where the additional subscript E indicates the country); (ii) infection outside the United Kingdom was rare (i.e. $p_{I,E}(t_O) << 1$); and (iii) infection was predominantly feed-borne, allows the following approximation:

$$\int_a^\infty \int_O^a \rho_C(a', u \mid t_O)\mathrm{d}a'\mathrm{d}u = p_{I,E}(t_O)\int_a^\infty h(u)\mathrm{d}u. \quad (2.17)$$

to be made where $h(u)$ is the convolution of the age-specific susceptibility/exposure distribution and the incubation period distribution such that

$$h(u) = \int_O^u g(a)f(u' - a)\mathrm{d}u'. \quad (2.18)$$

Thus, for animals born at time t_O the log likelihood of the EU testing data can be written as in equation (2.16) but with

$$Z_I(a|t_O) \cong -B(t_O)\frac{\mathrm{d}S(a)}{\mathrm{d}a}p_{I,E}(t_O)\int_a^\infty h(u)\mathrm{d}u \quad (2.19)$$

and

$$Z_{ID}(a|t_O) \cong -B(t_O)\frac{\mathrm{d}S_U(a)}{\mathrm{d}a}p_{I,E}(t_O)\int_a^\infty \psi(u - a)h(u)\mathrm{d}u. \quad (2.20)$$

For country E, the maximum-likelihood estimate for $p_{\mathrm{I},E}(t_0)$ is given by the closed form solution:

$$p_{\mathrm{I},E}(t_0) = \frac{x_{t_0,E}(a) - (1-\xi)n_{t_0,E}}{n_{t_0,E}(a)\left(\int_a^{\infty}\psi(u-a)h(u)\,\mathrm{d}u - (1-\xi)\right) + x_{t_0,E}(a)\int_0^a h(u)\,\mathrm{d}u}, \tag{2.21}$$

conditional upon the parameter values assumed for ξ, $\psi(w)$ and $h(u)$.

Because the ages of animals that tested negative were not available, it was necessary to estimate the distribution of birth dates among tested animals for each country using the survival function, $S(a)$, such that if N_E is the total number of apparently healthy animals tested in country E at time t, then the number tested between the ages of a_1 and a_2 is estimated to be $N_E(S(a_1) - S(a_2))/S(a_{\mathrm{L}})$ where a_{L} is the lower limit beyond which all apparently healthy animals slaughtered are tested for infection. The survival function estimated from data on British cattle was used in each case.

(c) *Demographic analysis*

Although analysis of cattle demography was not a primary objective of this study, investigation of the stability of the British cattle population was necessary to evaluate the adequacy of the assumption of a stationary survival function. We examined the demographic stability of the British cattle population by analysing the age distribution of cattle in 2001 and data on the slaughter of cattle for consumption since 1988. In both cases we determine the proportion of variation in the recent data explained by the predictions based on earlier data.

The survival probability as a function of age, $S(a)$, was previously estimated for British cattle from data obtained from the National Milk Records on the age distributions in a subset of dairy herds in 1982, 1988, 1989, 1991 and 1994 with at least 2000 cattle in each sample (Donnelly *et al.* 1997*b*; Donnelly & Ferguson 2000). For this updated analysis, we compare the observed age distribution of the national herd of cattle in GB on 1 July 2001 to that predicted using the survival function estimated in earlier work.

Data on the number and type of cattle slaughtered for consumption in the UK by week from 1988 were analysed to investigate seasonality in slaughter rates and stability of these patterns from year to year. Due to the impact of measures implemented to control and eradicate foot and mouth disease in 2001, we use a model fitted to the 1988–2000 data and use it to predict the patterns observed in the 2002 data.

A model for the weekly national slaughter rate was constructed allowing for the effects of cattle type and week of the year (1 to 53), allowing for overdispersion through the use of a log-normal likelihood function. Four additional parameters were included: three allowing for decreased slaughter rates following the 1996 public announcement linking new variant Creutzfeldt–Jakob Disease (vCJD) to BSE (permanently affecting rates for cows and adult bulls, but affecting other types for only four weeks) and one allowing for the sudden increase in the rate of slaughter of calves in August 1999.

(d) *vCJD prediction*

The potential size of the human vCJD epidemic in GB was investigated, as in earlier work (Ghani *et al.* 1998, 2000; Ferguson *et al.* 2002), conditioning on estimated past exposure to BSE-infected cattle but fitted to data on observed vCJD mortality. Through numerical solution of the inverse problem, the transmission coefficient was calculated as a function of case incidence, allowing incidence of vCJD deaths in any time interval to be treated as a model parameter. This enabled nonlinear optimization techniques (Press *et al.* 1992) to be used to obtain likelihood profiles (Cox & Medley 1989) by fitting the model to the joint age- and time-stratified mortality data (Ferguson *et al.* 2002). 95% confidence intervals (CI) were obtained using likelihood ratio tests.

3. DATA

The data on the age distribution of the British cattle population on 1 July 2001 were obtained from the Cattle Tracing System (CTS). Launched in September 1998, this system is administered by the British Cattle Movement, part of DEFRA. There is more detail in the system about cattle born since 1 July 1996, required by law to have cattle passports describing their life history. Older cattle have been issued with certificates of CTS registration, but their data are less complete with estimated dates of birth in many cases. Of the 9 707 226 cattle, 94% had age information with the remaining 6% being known to be over 5 years of age.

Data on the number of British cattle slaughtered for consumption by type (calves, heifers, cows, steers, young bulls and adult bulls) and week of slaughter were obtained from DEFRA (DEFRA 2002), relating to over 41 million cattle slaughtered between 1988 and June 2002 (figure 1*a*).

Data regarding each confirmed case of BSE arising in GB have been entered into a database maintained at the Veterinary Laboratories Agency (see Donnelly *et al.* (1997*b*) and Donnelly & Ferguson (2000) for additional details). The variables considered in the following analyses include date of birth and date of onset of the clinical signs of disease as well as the estimated age of the animal at clinical onset, used if the dates of birth and/or onset are unknown. As noted previously (Ferguson *et al.* 1997), until late 1990 when the farmer estimated the age at clinical onset, it was biased towards whole years of age. This bias was corrected for by resampling the ages of *ca.* 5000 cases by randomizing uniformly the month of the reported age at onset, as in earlier analyses (Ferguson *et al.* 1997).

The first two surveys of apparently healthy cattle in GB were mainly targeted at animals over 5 years of age. One survey, conducted between January and March 1999, detected 18 positives in 3945 cattle with test results. The second, conducted between May and December 2000, detected 42 positives in 10 032 cattle with test results.

Birth cohort data were available for the healthy cattle over 30 months of age slaughtered for consumption and tested in the EU between January and October 2001 (with the exception of October 2001 data for Ireland, Italy and The Netherlands). Table 1 presents the results stratified by birth cohort, where a birth cohort is defined as animals born from July of the preceding year to June of the year being considered. The number of animals tested is only stratified by country of origin because age information was only available for the animals with positive test results.

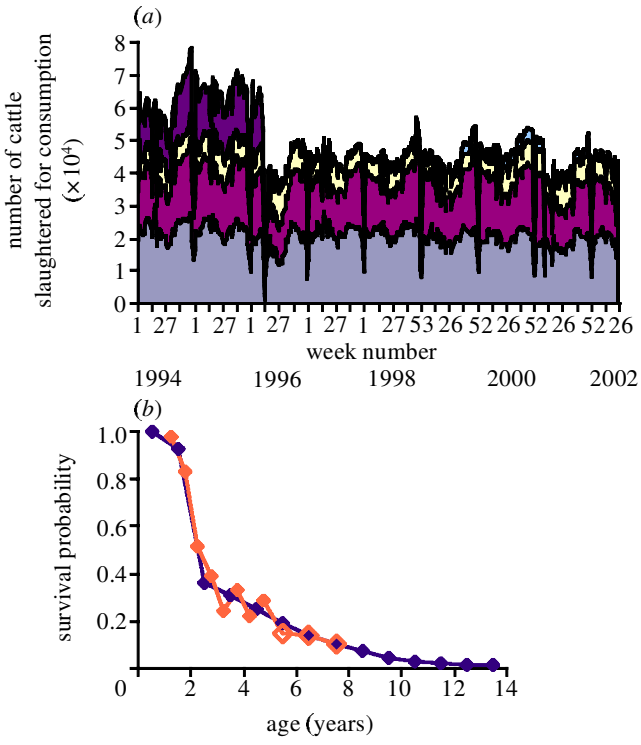

Figure 1. (*a*) Weekly number of cattle slaughtered for consumption in the UK by type (light purple, steers; dark pink, heifers; yellow, young bulls; green, calves; dark purple, cows; adult bulls are not visible on this scale). (*b*) Survival curves estimated from the 2001 age distribution of cattle (red) and previously estimated from independent data (blue) (Anderson *et al.* 1996; Donnelly *et al.* 1997*b*). The open red diamonds differ from the solid red diamonds in that because 6% of the cattle population were recorded with unknown ages over 5 years, these survival estimates based only on those animals with ages recorded as 5, 6 or 7 years may be underestimates.

4. RESULTS

(a) *Demography*

The survival probabilities estimated from the age distribution in July 2001 provided a remarkably good fit to the previously estimated survival function (Anderson *et al.* 1996; Donnelly *et al.* 1997*b*; figure 1*b*). A survival function must by definition be monotonically decreasing, so the small oscillations observed in the new estimates between the ages of 2.5 and 5 years are most probably due to seasonal fluctuations in farming practices. Further work will examine multiple snapshots of the age distribution of the national herd to provide insight into this source of variation and to investigate possible effects of the foot and mouth epidemic on cattle demography.

The model fitted to the slaughter rate data explained 72.1% of the weekly variation in the 4068 observed rates (678 weeks of data for six cattle types). Furthermore, this model explained 70.9% of the week-to-week variation observed in the 2002 slaughter data. The key changes in the slaughter patterns were both in response to changes in British agricultural policy: (i) the sudden end to the slaughter of cows and adult bulls for consumption following the ban on OTM cattle in 1996; and (ii) the dramatic increase in the rate of calves being slaughtered for consumption from August 1999 following the discontinuation of the Calf Processing Aid Scheme. The scheme had compensated farmers for male calves slaughtered before 20 days of age provided that the product of slaughter did not enter the human food chain.

It is not surprising that these changes in the cattle slaughter practices in recent years did not affect the survival distribution, because it summarizes mortality from all causes (slaughter for consumption, slaughter under a compensation scheme and natural causes). The age distribution and slaughter data demonstrate stability in the demography of the cattle population. Thus, the use of a

Table 1. Test results from January to October 2001 for apparently healthy cattle slaughtered over 30 months of age by country of origin and birth cohort.

	number of positive tests by birth cohort							
	pre-1994	1994	1995	1996	1997	1998	total	number of cattle tested
Austria	0	0	0	0	0	0	0	176 028
Belgium	2	3	4	10	5	0	24	280 510[b]
Denmark	0	0	0	1	0	0	1	202 922[b]
Finland	0	0	0	0	0	0	0	5124
France	0	6	25	23	2	1	57	1 878 519
Germany	2	0	7	16	4	1	30	2 091 530
Greece	0	0	0	0	1	0	1	12 068
Ireland[a]	6	4	3	5	0	0	18	315 668
Italy[a]	0	2	2	7	7	0	18	225 870
Luxembourg	0	0	0	0	0	0	0	19 134
Netherlands[a]	0	1	1	3	1	0	6	289 904
Portugal	2	2	2	2	0	0	8	16 666
Spain	6	10	4	6	1	1	28	249 920
Sweden	0	0	0	0	0	0	0	2176

[a] For Ireland, Italy and The Netherlands results are from January to September 2001.
[b] The number of cattle tested obtained is given in all cases as the sum of the reported monthly figures. For Belgium and Denmark this total was inconsistent with the cumulative total reported on the Web site. However, the difference was less than 0.05% in both cases.

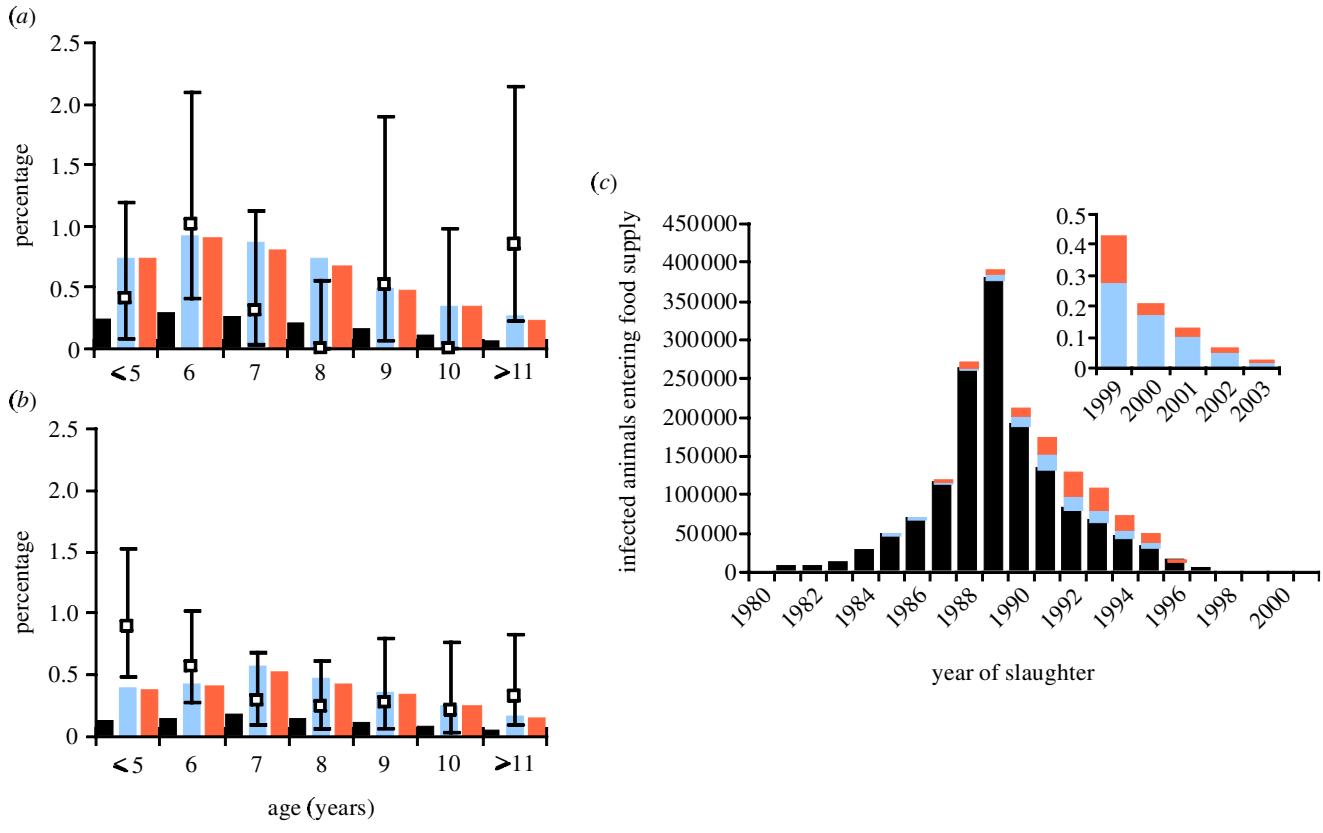

Figure 2. Estimated age-specific prevalence of infection (with exact 95% CI) for (*a*) the 1999 and (*b*) 2000 surveys (open squares), and expected prevalence from the back-calculation models. Back-calculation model results are shown separately for the no underascertainment (black bars), differential mortality (blue bars), and underreporting scenarios (red bars). The ages of cattle without age data, all with negative test results, were assumed to be distributed like those animals with year of age but no date of birth recorded. (*c*) Estimated exposure to BSE-infected cattle over the course of the epidemic from the model with differential mortality (unreported cases or differentially slaughtered animals, red; animals slaughtered in last year of incubation, blue; animals slaughtered before last year of incubation, black). It is unclear what proportion of unreported clinical cases entered the food supply, although it may have been substantial if preferentially slaughtering of preclinical animals (differential mortality) was common. Inset shows estimated exposure in recent years from animals in the final year of BSE incubation, allowing for the impact of the OTM ban. All results shown assume complete test sensitivity in the last 10% of the incubation period, although results for the differential mortality model are very similar irrespective of the assumed sensitivity function.

constant survival function over the course of the epidemic is justified.

(b) *GB infection prevalence*

In the British OTM surveys the age distribution in the tested cattle with age data available, 2219 in 1999 and 8990 in 2000, was similar to that predicted for slaughtered cattle using the estimated survival function, $S(a)$. The geographical and breed distributions of the 60 test-positive animals were not significantly different from those observed in clinical cases with 1999 and 2000 onsets ($p = 0.82$ and $p = 0.71$, respectively). Thus, there was no evidence of bias in the selection of individual animals to be tested.

Assuming the diagnostic procedure employed was fully specific and fully sensitive for the last 10% of the incubation period, the age-specific infection prevalence estimates (figure 2*a*,*b*) from the British OTM testing data were substantially higher than predicted by back-calculation models of clinical cases in GB which assumed complete ascertainment of clinical cases after mid-1988 when BSE became a notifiable disease (i.e. $\Lambda(t) = 1$ after mid-1988 and $\kappa(w) = 0$ for all w throughout the epidemic). In both cases an additional parameter for the rate of maternal transmission in the last six months of the maternal incubation period was estimated as a parameter of the model.

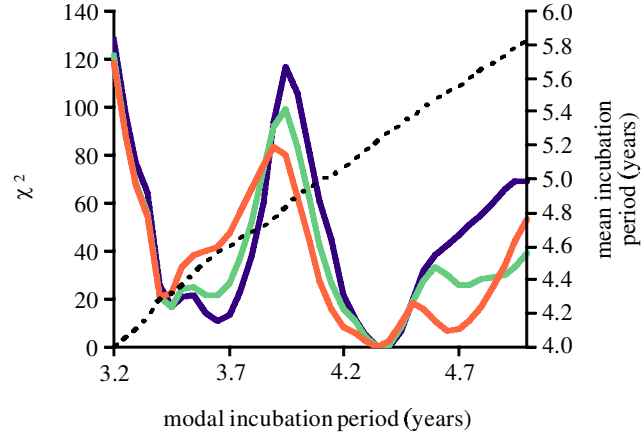

Figure 3. The sensitivity of the goodness-of-fit model to the modal and mean incubation period is displayed for models allowing for underreporting of clinical cases and complete test sensitivity in the last six months (blue), 1 year (green) and 3 years (red) of the incubation period.

Mechanisms leading to underascertainment of clinical

cases of BSE were explored through the fitting of a single additional parameter, representing either the proportion of cases reported following mid-1988, λ, or the proportion of infected animals that survive to disease onset but are slaughtered just before clinical signs become apparent, K. The model fit as measured by deviance improves by more than 60 ($p < 0.001$) under varied assumptions regarding test sensitivity (table 2). The probability of maternal transmission was fitted in each case, assuming such transmission only occurs in the last six months of the maternal incubation period.

This analysis substantially increases the estimated scale of the BSE epidemic in GB, with 3.5 (95% CI: 2.6, 4.4) million infections and 2.9 (2.2, 3.6) million infected cattle (excluding 0.42 (0.27, 0.56) million non-ascertained clinical cases) slaughtered for consumption by mid-2001 in the model assuming ongoing underreporting and complete test sensitivity in the last 10% of the incubation period. The comparable estimates for the differential mortality model are 1.9 (1.6, 2.3) million infections, 1.6 (1.3, 1.9) million infected animals slaughtered and 0.16 (0.11, 0.22) million non-ascertained cases. This compares with 1.05 (0.97, 1.12) million infections and 0.87 (0.80, 0.94) million infected animals slaughtered assuming complete case ascertainment from mid-1988 onwards. Projections for future reported clinical cases (by year of clinical onset) are 580 (95% prediction interval: 500–740) for 2001, 230 (190–350) for 2002, and 80 (65–150) for 2003 for the differential mortality model (projections are virtually identical for the underreporting model, and vary little as a function of assumed test sensitivity).

In the model with ongoing underreporting of cases, the results obtained assuming the test is completely sensitive in a fixed proportion of the incubation period are similar to those obtained assuming sensitivity in the last 3 years of the incubation period. One might have naively expected assuming sensitivity in the last 10% of the incubation period would be similar to assuming sensitivity in the last six months, because the incubation period is on average 5 years. However, because the animals tested were all relatively old, their mean incubation period is considerably more than 5 years, making the last 10% substantially greater than six months.

In the differential mortality model, assumptions about the duration of complete sensitivity had little impact, as all differential mortality was assumed to be concentrated in the period just prior to when clinical onset would have been expected. Hence, irrespective of the duration of complete sensitivity, the infection status of these animals would be accurately detected by the test used. If differential mortality were more widely distributed over the course of the incubation period, then incubation-stage-dependent test sensitivity would have had more impact on the fit of the model.

(c) *Specificity*

An alternative explanation for increased prevalence in the OTM studies, compared with that predicted by the observed incidence of clinical cases, is that false positives are being detected. The models assuming no underreporting or differential mortality, but allowing for incomplete test specificity, achieved the best fits to the data (deviance values of between 601.6 and 603.0, depending on the assumption about sensitivity) with specificity estimates of between 99.60 and 99.70% and 95% CIs of width 0.2 to 0.3%.

The consistency of the test results (of the 42 positive animals in the 2000 survey, 39 had BSE-specific changes in the brain detected by histopathological examination, two had inconclusive histopathology but positive Western blot and immunohistochemistry test results, and one had negative histopathology but positive DELFIA and immunohistochemistry test results; DEFRA 2001*a*), indicates that the overall specificity of the test protocol must have been quite high. The primary test used by DEFRA for cattle slaughtered as BSE suspects is histopathology and is considered the gold standard. Western blot and immunohistochemistry tests are also routinely used. If the testing of EU animals had a specificity of 99.70%, even if the true prevalence were zero the tests would have identified over 17 000 (false) positives. Thus, although the inclusion of incomplete specificity can improve the model fit to the British data, such low levels are clearly improbable given other data.

(d) *Incubation period*

Back-calculation analysis of clinical case data previously estimated the mean incubation period of BSE to be between 4.75 and 5 years (Ferguson *et al.* 1997). The current integrated analysis gave a higher estimate, 5.2 years (5.1, 5.3) (figure 3), with the relationship between the mean and the modal incubation period being independent of the duration of test sensitivity assumed and whether the differential mortality or underreporting model was used. The presence of local minima in the deviance profile for modal incubation period shown in figure 3 highlights the importance of rigorous numerical optimization in model fitting.

As discussed in earlier work (Ferguson *et al.* 1997), it is possible that over the course of the BSE epidemic the mean incubation period varied. For example, a decrease in the average infectious dose received by cattle in the 1990s might have lengthened the incubation period, given evidence that cattle experimentally infected through oral dosing exhibit shorter incubation periods if they receive larger infectious doses (Anderson *et al.* 1996). However, extrapolating from rodent models (Weissmann 1991), serial passaging of BSE within the cattle population might have shortened the incubation period over the course of the epidemic (for a given infectious dose). Comparison of the observed and fitted distribution of ages at clinical onset (figure 4) gives little indication of any changes in the incubation period of the disease, given the qualitatively good fit of the model with fixed incubation period distribution shown. We did not therefore explore the effect of temporal variation in the incubation period further here. However, future analyses should monitor the situation and investigate any evidence of such changes.

(e) *Maternal transmission*

From November 1998 the European Commission required the culling of the offspring of confirmed BSE cases as a precondition for the decision for a date based export scheme. The UK was required to slaughter all offspring born after 1 August 1996 to confirmed BSE cases (including the offspring of cases confirmed prior to the

Table 2. Maximum-likelihood estimates and deviances for two mechanisms of underascertainment: ongoing underreporting and differential mortality.
(Underascertainment estimates are provided for each model: for the underreporting model $1-\lambda$ assuming that $\Lambda(t)=\lambda$ after mid-1988; and for the differential mortality model K assuming that a fraction K of infected animals that survive to disease onset are slaughtered just before clinical signs become apparent. The test is assumed to be completely specific in all cases and completely sensitive in the latter portion of the incubation period (either defined as a fixed interval or as a proportion of the animal's incubation period).)

	complete ascertainment			underreporting			differential mortality			
portion of the incubation period with complete test sensitivity	deviance	estimate (95% CI) total infection incidence (millions)	deviance	$1-\lambda$	estimate (95% CI) total infection incidence (millions)	maternal transmission rate[a] (%)	deviance	K	estimate (95% CI) total infection incidence (millions)	maternal transmission rate[a] (%)
proportion										
0.1	674.9	1.05 (0.97, 1.12)	612.9	0.70 (0.60, 0.76)	3.50 (2.64, 4.37)	0.5 (0, 2.8)	607.7	0.46 (0.36, 0.54)	1.94 (1.64, 2.28)	0.5 (0, 2.4)
0.2	674.9	1.05 (0.97, 1.12)	613.0	0.70 (0.60, 0.76)	3.50 (2.65, 4.37)	0.5 (0, 2.8)	607.7	0.46 (0.36, 0.54)	1.94 (1.64, 2.28)	0.5 (0, 2.4)
0.6	675.5	1.05 (0.97, 1.12)	613.1	0.70 (0.60, 0.76)	3.50 (2.65, 4.47)	0.5 (0, 2.8)	607.8	0.46 (0.36, 0.54)	1.94 (1.64, 2.28)	0.5 (0, 2.4)
duration (years)										
0.5	807.9	1.05 (0.97, 1.12)	643.8	0.86 (0.84, 0.88)	7.98 (6.66, 9.18)	1.2 (0, 3.8)	608.7	0.58 (0.50, 0.64)	2.50 (2.10, 2.93)	0.6 (0, 2.6)
1	747.1	1.05 (0.97, 1.12)	625.1	0.83 (0.78, 0.86)	6.22 (4.81, 7.64)	0.9 (0, 4.3)	608.4	0.54 (0.46, 0.61)	2.28 (1.94, 2.70)	0.6 (0, 2.6)
3	687.7	1.05 (0.97, 1.12)	614.4	0.73 (0.64, 0.79)	3.94 (2.97, 5.02)	0.6 (0, 3.0)	607.9	0.48 (0.38, 0.56)	2.02 (1.69, 2.39)	0.5 (0, 2.4)

[a] These estimates have not been adjusted for the effect of the offspring cull. See § 4e for further details.

decision). The initial backlog was cleared in June 1999 and the policy is ongoing. Because maternal transmission is assumed to be limited to the last six months of the incubation period of the dam, assuming complete case ascertainment the offspring cull would, if perfectly implemented, have prevented nearly all of the clinical cases expected to arise from late 1998 among maternally infected cattle. If there was underascertainment, however, the offspring cull would only prevent a proportion, either K or $1-\lambda$, of these offspring cases because only the offspring of ascertained cases would be culled.

Full incorporation of the effects of the offspring cull into the back-calculation model is complex due to the need to track its effect through multiple generations of infection, and is the subject of ongoing work. Hence here we present naive estimates of the maternal transmission rate, not allowing for the offspring cull. However, from the argument above, these estimates can conservatively be adjusted to allow for the proportion of clinical cases among offspring prevented by the offspring cull through a multiplicative scaling of either $1/(1-\lambda)$ or $1/K$.

The probability of maternal transmission estimated from the BSE survey and clinical incidence data is 0.5% (0, 2.8%) in the last six months of the maternal incubation period in the model with ongoing underreporting of clinical cases and 100% test sensitivity in a fixed proportion of the incubation period (table 2). For the differential mortality model the corresponding estimate is *ca*. 0.5% (0, 2.4%) for all assumptions regarding test sensitivity. Allowing for the offspring cull, and using the correction factors discussed above, these estimates increase to *ca*. 0.7% (0, 4.0%) and 1% (0, 5.2%), respectively. The level of maternal transmission is only now estimable using back-calculation methods because in recent years the risk of feed-borne infection is thought to have been virtually eliminated.

Even allowing for the possible impact of the offspring cull, these estimates are substantially lower than the 10% value conservatively examined in past work based on the maternal cohort study (Donnelly *et al.* 1997*c*), but remain consistent with estimates from analysis of dam–calf pairs of BSE cases (Donnelly *et al.* 1997*a*). The analysis of the maternal cohort study data provided estimates of the probability of maternal transmission and the duration of maternal infectiousness. Scaling the duration to equal six months, the estimates were comparable to 5–9% depending on assumptions (Donnelly *et al.* 1997*c*). The study of dam–calf pairs produced estimates of *ca*. 2–3% for the last six months of the maternal incubation period (Donnelly *et al.* 1997*a*).

(f) *Consequences for human exposure*

These results do not increase the upper bound on the predicted size of the human vCJD epidemic in GB (Ghani *et al.* 1998, 2000; Ferguson *et al.* 2002), since published analyses conditioned on estimated past exposure to BSE-infected cattle (figure 2*c*) but fitted to data on observed vCJD mortality. For consistency between BSE exposure data and vCJD case incidence, increased estimates of past exposure result in lower estimates of the infectiousness of bovine material to humans. Furthermore, the updated estimate of late-stage infected animals slaughtered for consumption under 30 months in 2000 is less then one per

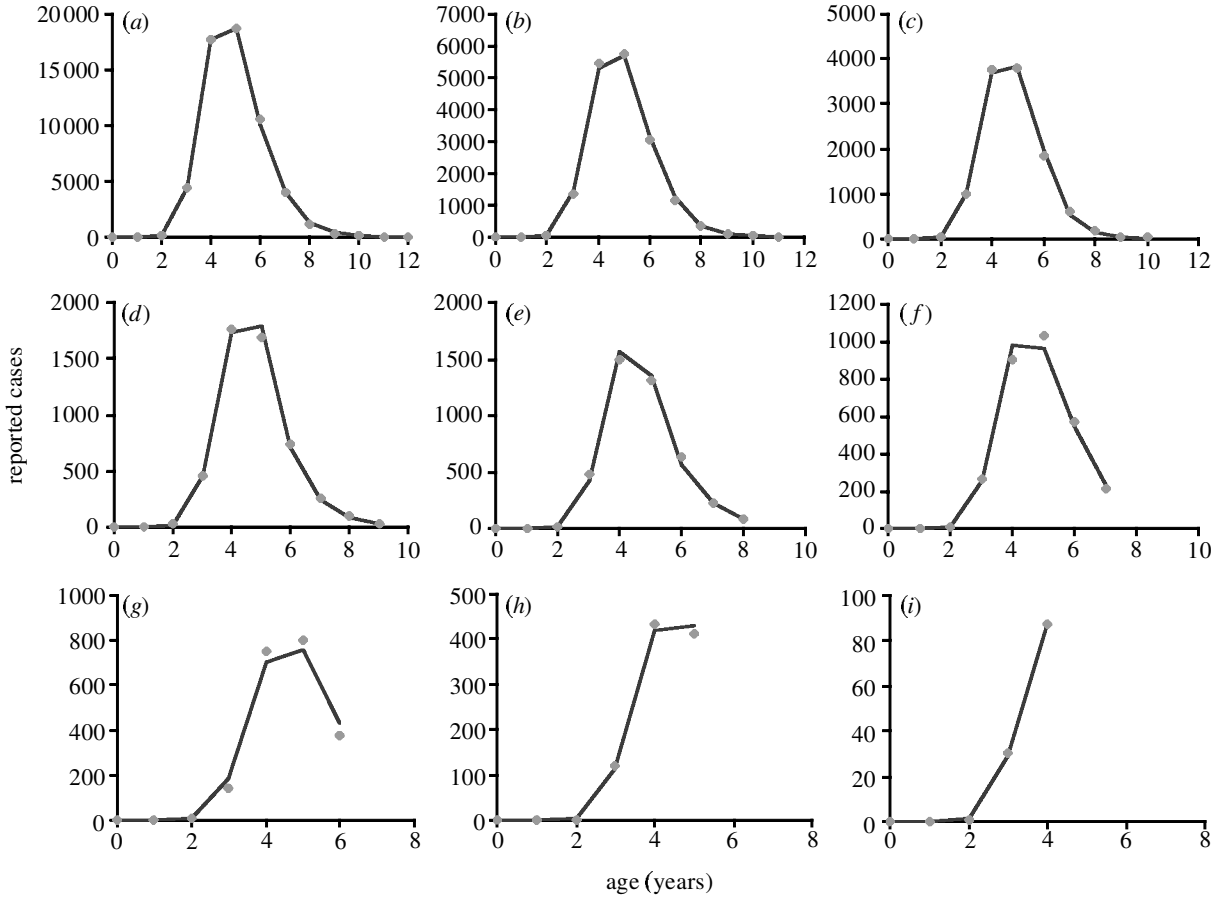

Figure 4. The observed (diamonds) and fitted (line) number of confirmed BSE cases by birth cohort and age (in years) at onset for cohorts from the model with differential mortality and test sensitivity in the last 10% of the incubation period. (*a*) 1988, (*b*) 1989, (*c*) 1990, (*d*) 1991, (*e*) 1992, (*f*) 1993, (*g*) 1994, (*h*) 1995 and (*i*) 1996.

annum (figure 2*c*), in line with our earlier estimates (see Food Standards Agency 2000).

(g) *EU infection prevalence*

Per-head incidence of infection for other EU countries was estimated for the 1994, 1995, 1996 and 1997 birth cohorts (figure 5). As anticipated on the basis of the incidence of confirmed clinical cases, the infection incidence in the 1994 and 1995 cohorts was highest in GB. The rates across Europe were more similar in the 1996 and 1997 cohorts. However, it is noteworthy that despite the small number of clinical cases confirmed to date, the testing data indicate that infection incidence in the 1997 cohort was greater in Greece, Italy, Belgium, Spain and The Netherlands than in GB, the differences being highly significant for Italy and Belgium.

5. DISCUSSION

The OTM surveys represent an important independent dataset allowing underascertainment of BSE cases to be rigorously examined. Although the analysis is unable to determine definitively whether underreporting or differential mortality was the principal mechanism underlying the substantial level of underascertainment identified, the issues of key ongoing concern are the number of infected animals currently entering the human food supply and the potential size of the ongoing vCJD epidemic. Both of these are relatively insensitive to allowing for case underascertainment.

We considered other possible mechanisms that could have given rise to the higher than expected infection prevalence observed in both OTM surveys. It is unlikely that the prevalence seen is due to the presence of a class of infected animals that would never experience the onset of recognizable clinical signs of disease (i.e. with incubation periods greatly exceeding the natural lifespan of cattle), because of the 42 positive animals in the 2000 survey, 39 had BSE-specific changes in the brain detected by histopathological examination (DEFRA 2001*a*). Moreover, it is unlikely that the tests used are fully sensitive throughout the incubation period. For example, the Bio-Rad (CEA) test performed better than the DELFIA test (Moynagh *et al.* 1999) in EU evaluation but was unable to detect infection in animals early in the BSE incubation period (Grassi *et al.* 2001).

The different assumptions about test sensitivity explored in this paper (sensitive only for either a fixed amount of time at the end of the incubation period, or for a fixed proportion of the incubation period) mirror different possible mechanisms for the observed variation in the incubation period of BSE. Assuming that a test detects all animals with abnormal prion levels above a particular threshold (below the threshold associated with clinical signs of disease), then if prion density grows exponentially at the same rate in all infected animals we would expect

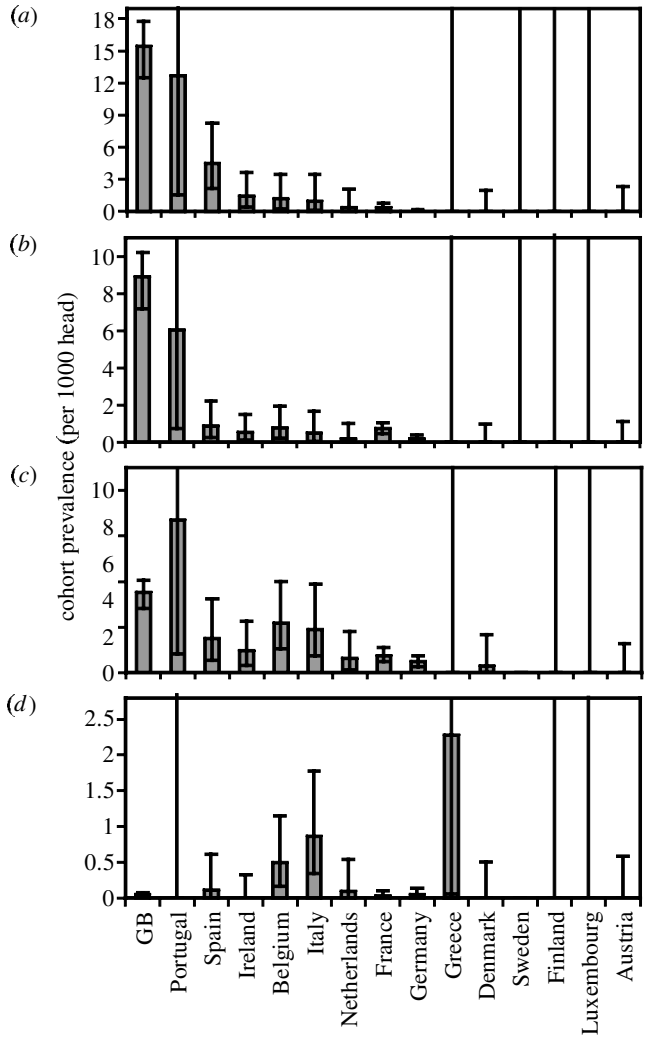

Figure 5. Country-specific estimated per-head infection incidence rates in the (*a*) 1994, (*b*) 1995, (*c*) 1996, and (*d*) 1997 birth cohorts (with 95% CI). The GB estimate was obtained from the integrated back-calculation analysis of clinical case data and OTM sample survey data. All other estimates were obtained solely from the analysis of testing data from apparently healthy animals slaughtered for consumption.

a fixed time interval of sensitivity at the end of the incubation period. Under this assumption of an invariant prion growth rate, it is still possible to explain the population variability in incubation period in terms of variation in initial dose. This conceptual model underlies the parametric form for the incubation period distribution first introduced by Medley and Short (Ferguson *et al.* 1997), and it is consistent with the relatively small variance observed in the incubation periods in cattle given identical doses in the experimental oral infection study (see Anderson *et al.* 1996).

Alternatively, it could be hypothesized that the observed natural variability in BSE incubation period was entirely attributable to between-animal variation in the rate of prion growth, with all cattle receiving identical doses. Then one would expect tests to be sensitive in a fixed proportion of the incubation period (leading up to clinical onset), again assuming that test sensitivity was dependent on prion density exceeding some threshold. However, there are no data to support this hypothesis, and it could be argued to be unlikely on biological grounds given the lack of any apparent genetic variability in susceptibility to infection among cattle (Hunter *et al.* 1994; Donnelly *et al.* 1997*a*).

If it is concluded that a fixed duration of test sensitivity at the end of the incubation period is the more realistic model, then the analysis of the OTM screening data presented here clearly indicate that differential mortality is a much more probable explanation for the observed underascertainment of cases than is underreporting. Possible causes for such preferential slaughtering of late stage infected animals without obvious clinical signs might include reduced milk yields or loss of weight, but this issue requires further investigation. Thus the most probable scale of the GB epidemic is that two million cattle were infected up to the present time.

However, both further analysis and additional data are needed, particularly if the conclusions of this study are to be used in informing policy. Future work will explore models fitting both differential mortality and underreporting, and examine more realistic functional relationships between test sensitivity and time to clinical onset in the tested animal (e.g. a sigmoidal sensitivity profile where partial sensitivity is seen during a portion of the incubation period). More importantly for the current study, we were not able to exclude the possibility of a sampling bias resulting in cattle from more heavily affected herds in a region being more likely to be tested, because the herds of origin were not identified for all tested animals, being only retrospectively determined for those found to be positive. Thus in future research it is critical that sample-based testing programmes (DEFRA 2001*b*) record the herd of origin and age data for every animal tested. Furthermore, the sensitivity and specificity of currently used screening tests need to be rigorously established for animals early in the incubation period of BSE. Information on infection prevalence in 'at-risk' groups—such as animals found dead on the farm, subject to emergency slaughter, or identified as sick at abattoirs—might allow assessment of the extent of preferential slaughter of preclinical infected animals. Additional data may also allow characterization of any temporal changes in underascertainment in recent years.

The results of this study demonstrate the effectiveness of the additional control measures implemented in GB in 1996 after the identification of vCJD. The analysis provides a standardized framework for the comparison of EU countries, illustrating the importance of evidence-based comparative risk assessment. Furthermore, this work indicates that countries such as Italy and Belgium, with few confirmed clinical cases but relatively high recent infection risks, may warrant additional control and enforcement measures to reduce future case numbers and avoid lengthening the time-course of the epidemic.

We thank John Wilesmith (Veterinary Laboratories Agency) and DEFRA for provision of BSE case data and the detailed OTM survey results, and the Food Standards Agency (Rosemary Hoffman specifically) for provision of data on the ages of apparently healthy test positive animals for each EU country. We also thank members of the Food Standards Agency OTM Review Group and the SEAC Epidemiology Subcommittee for stimulating discussions. We thank the Food

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